

Fig. 1

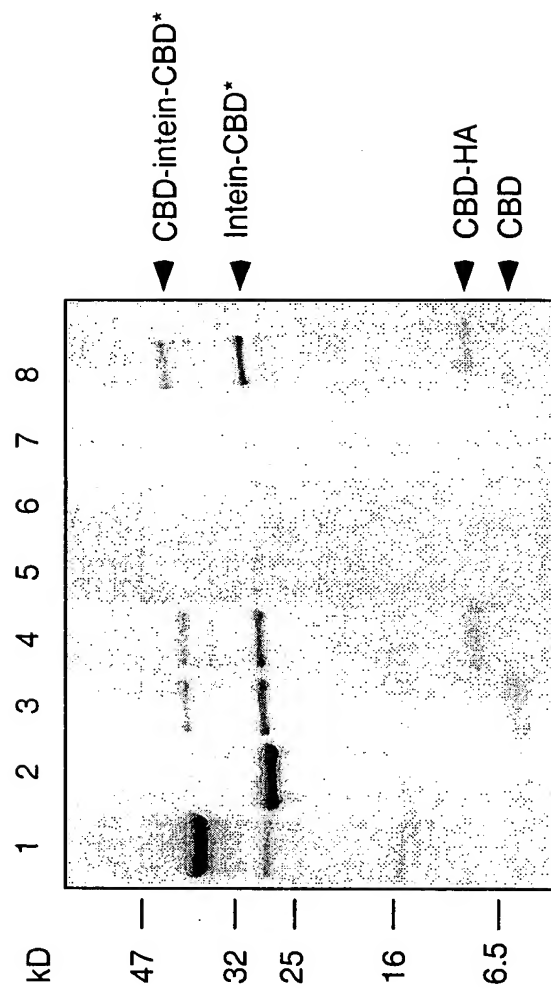


Fig. 2

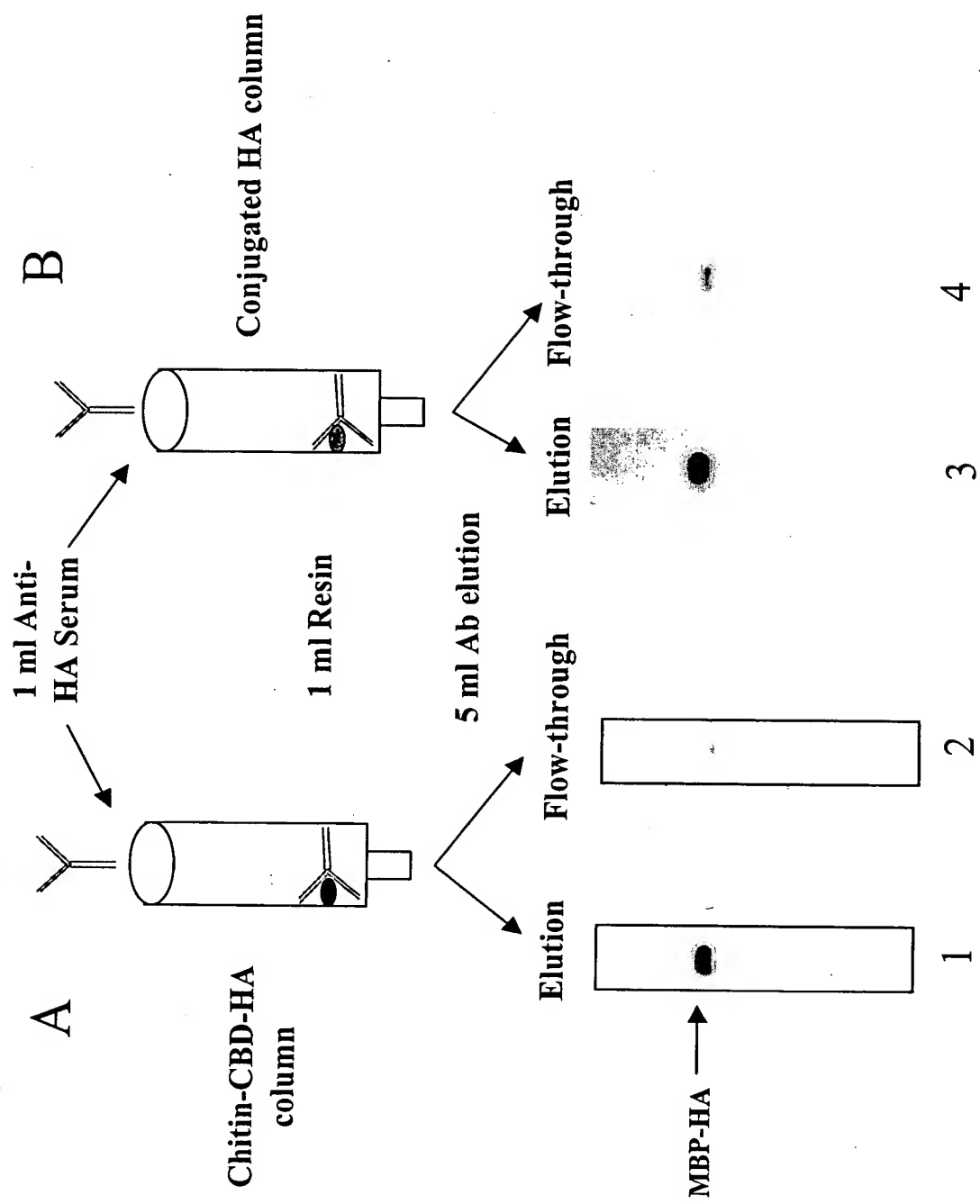


Fig. 3

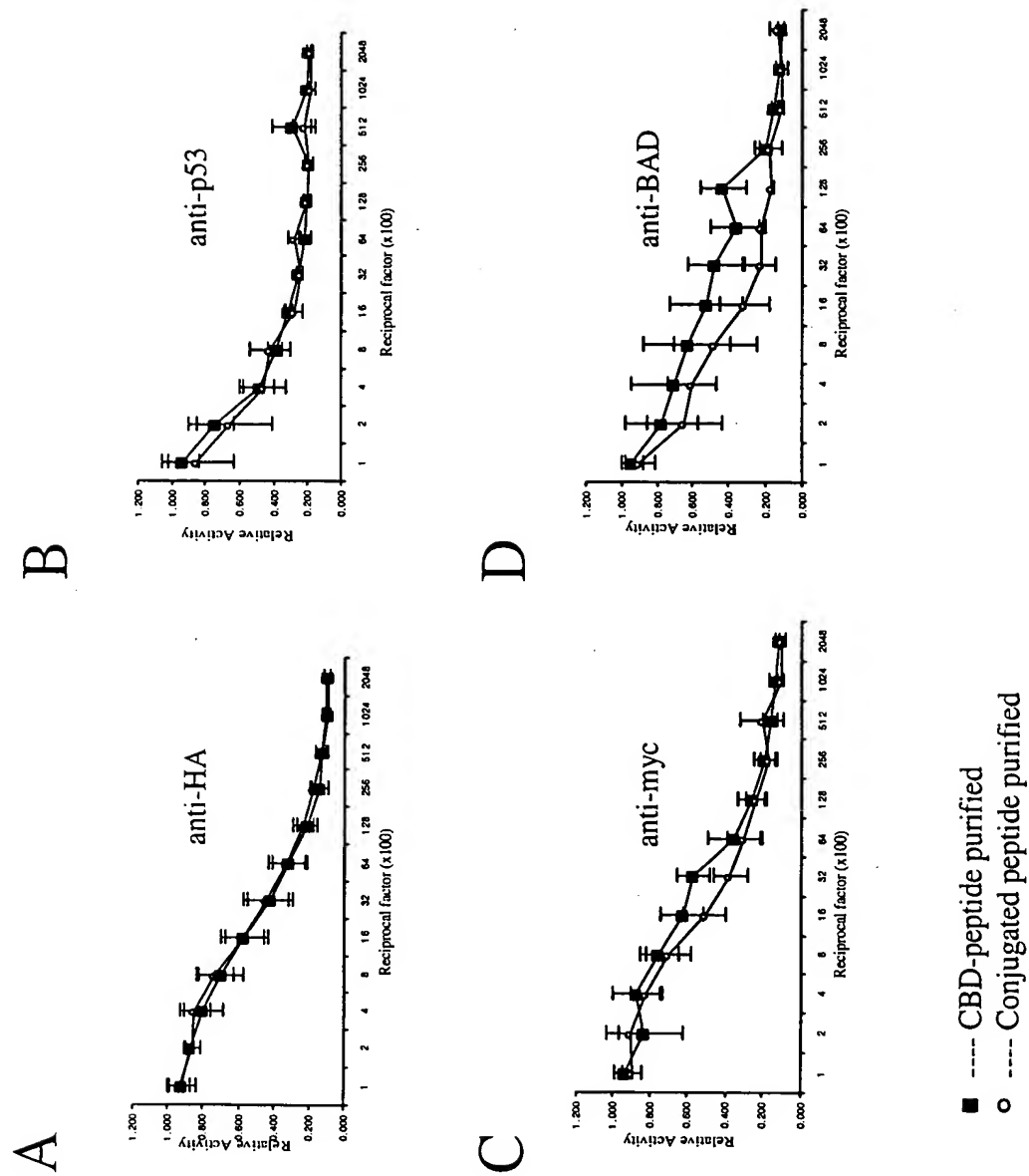


Fig. 4

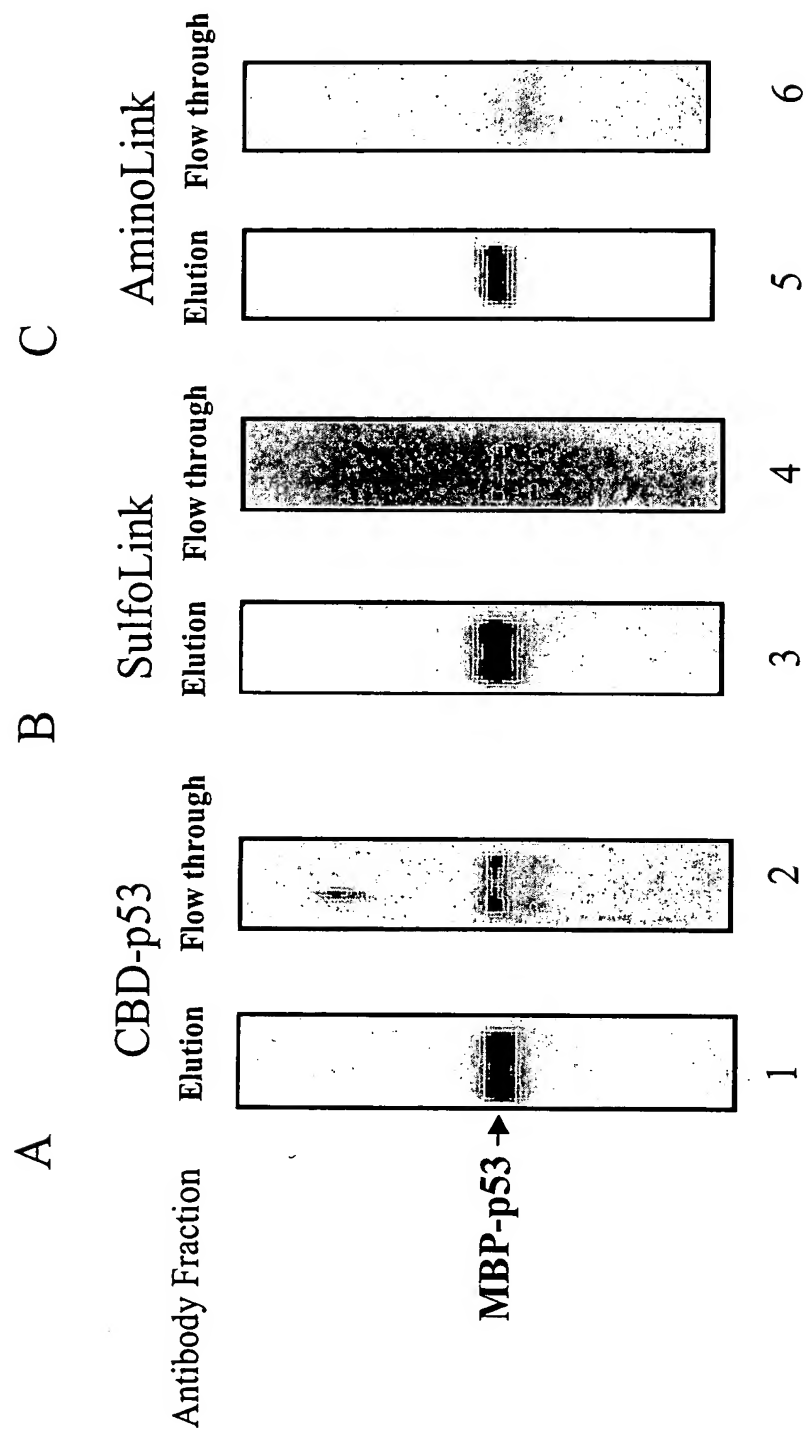
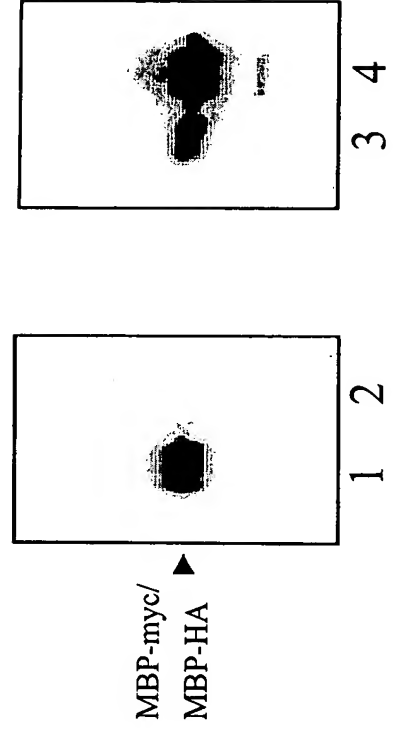
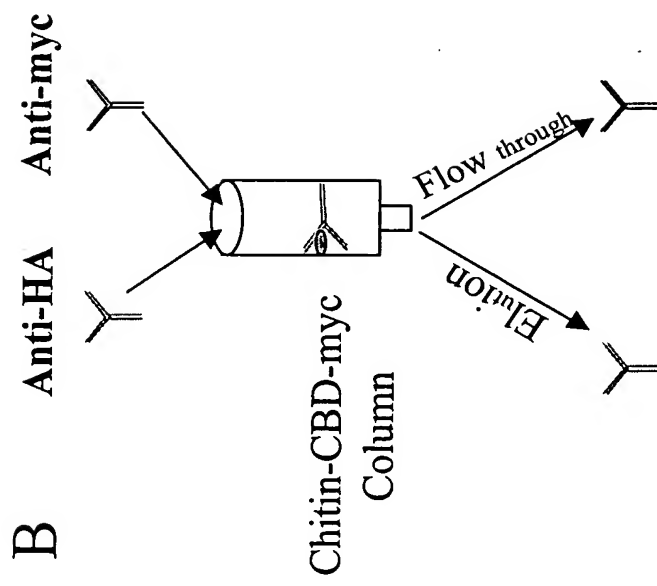
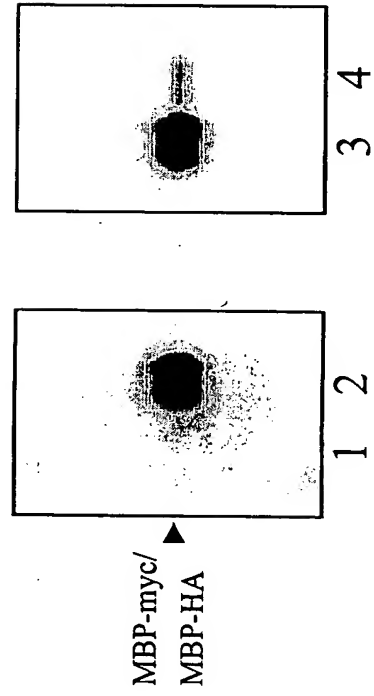
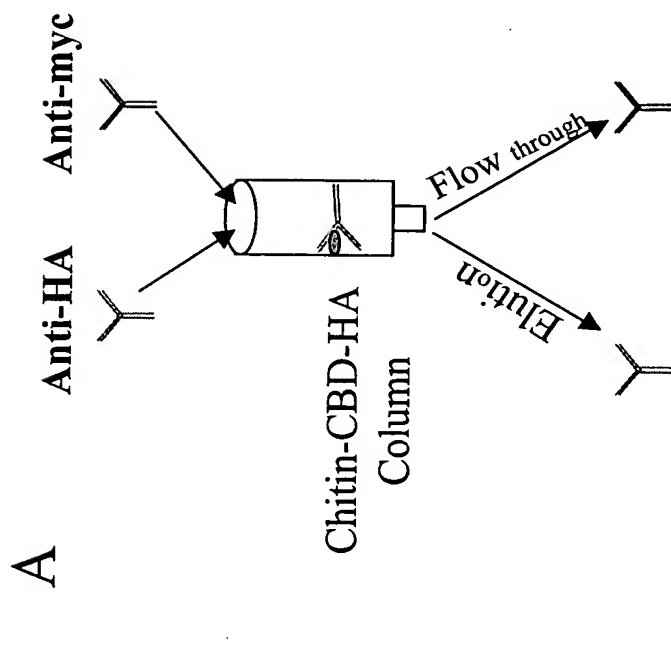


Fig. 5



**Fig. 6**

Figure 7A

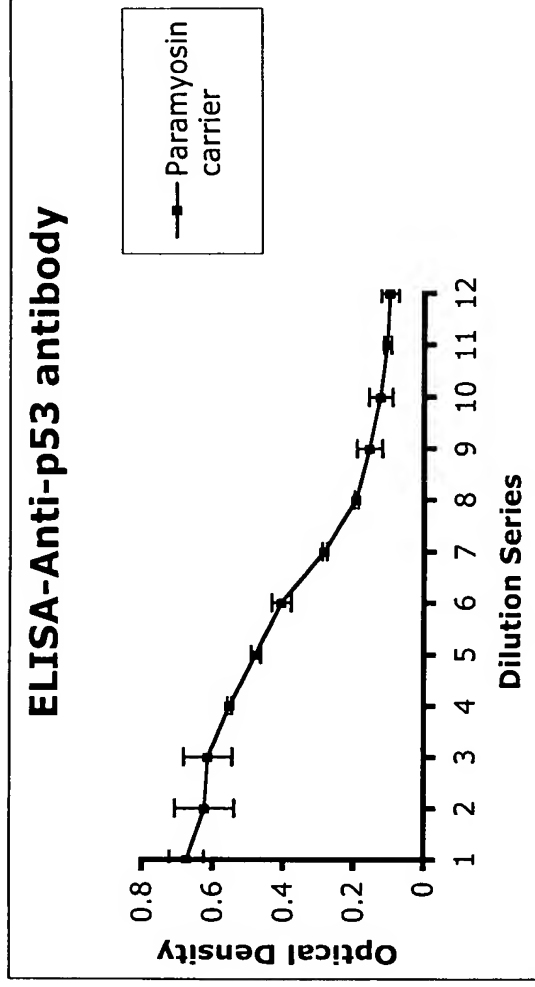


Figure 7B

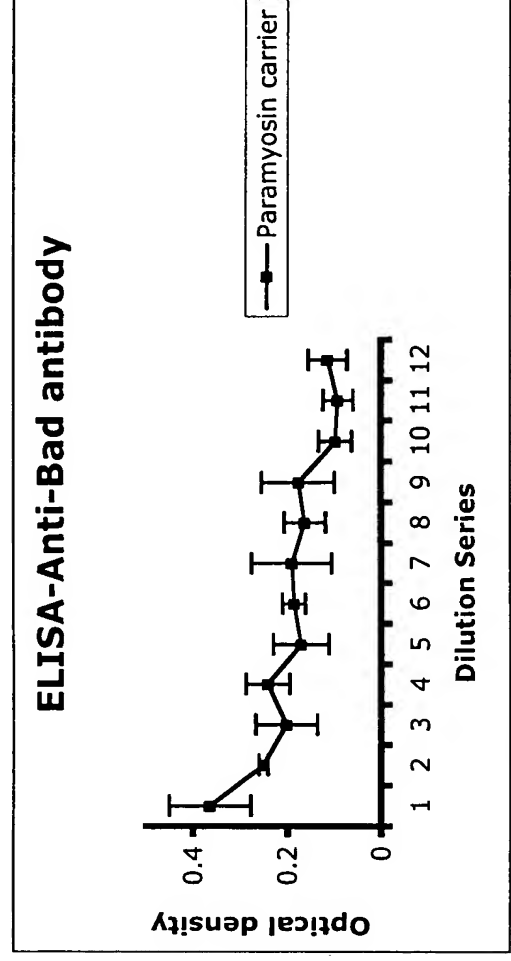


Figure 8

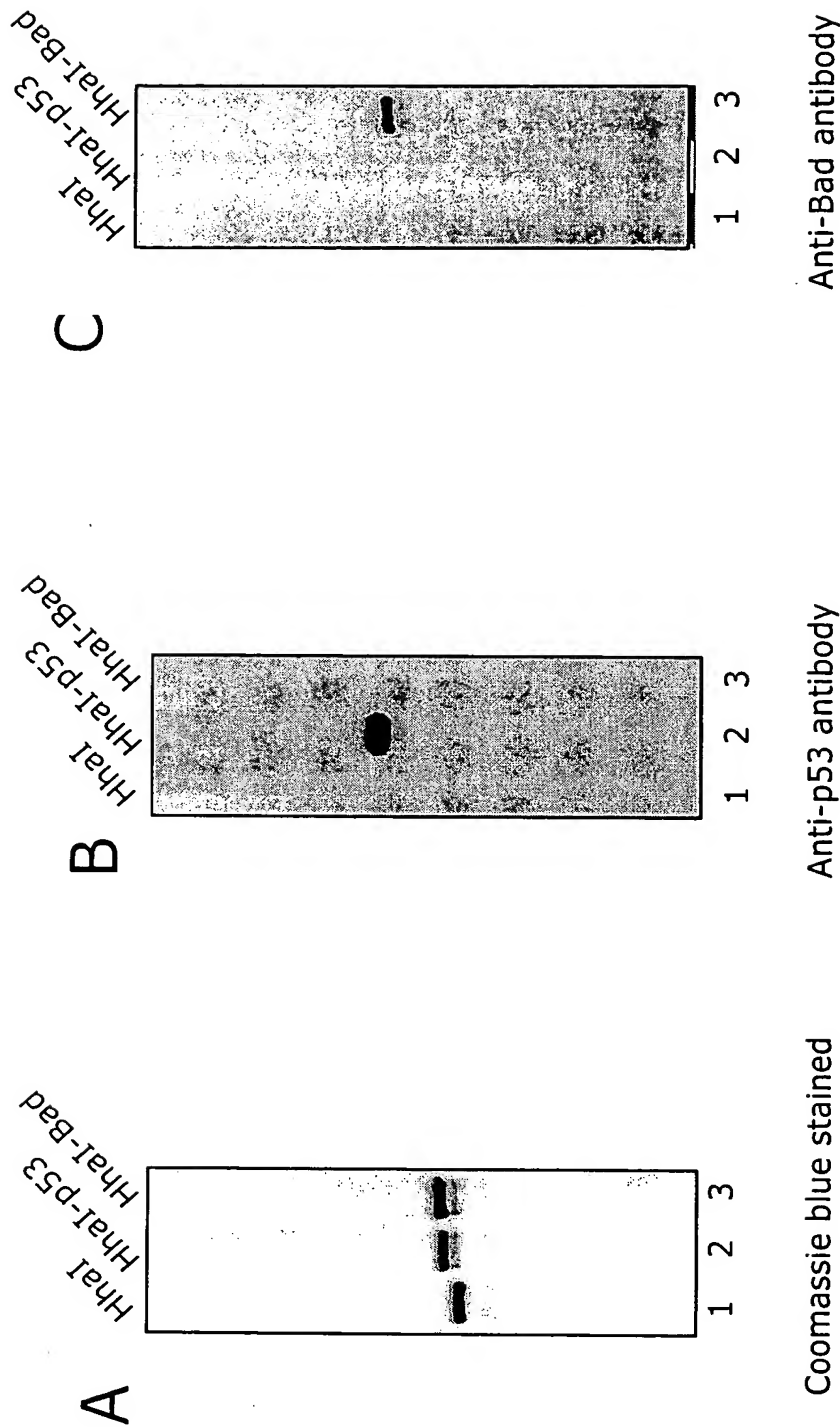




Figure 9. Flow chart of immobilizing peptide substrates  
onto membrane or other solid matrix

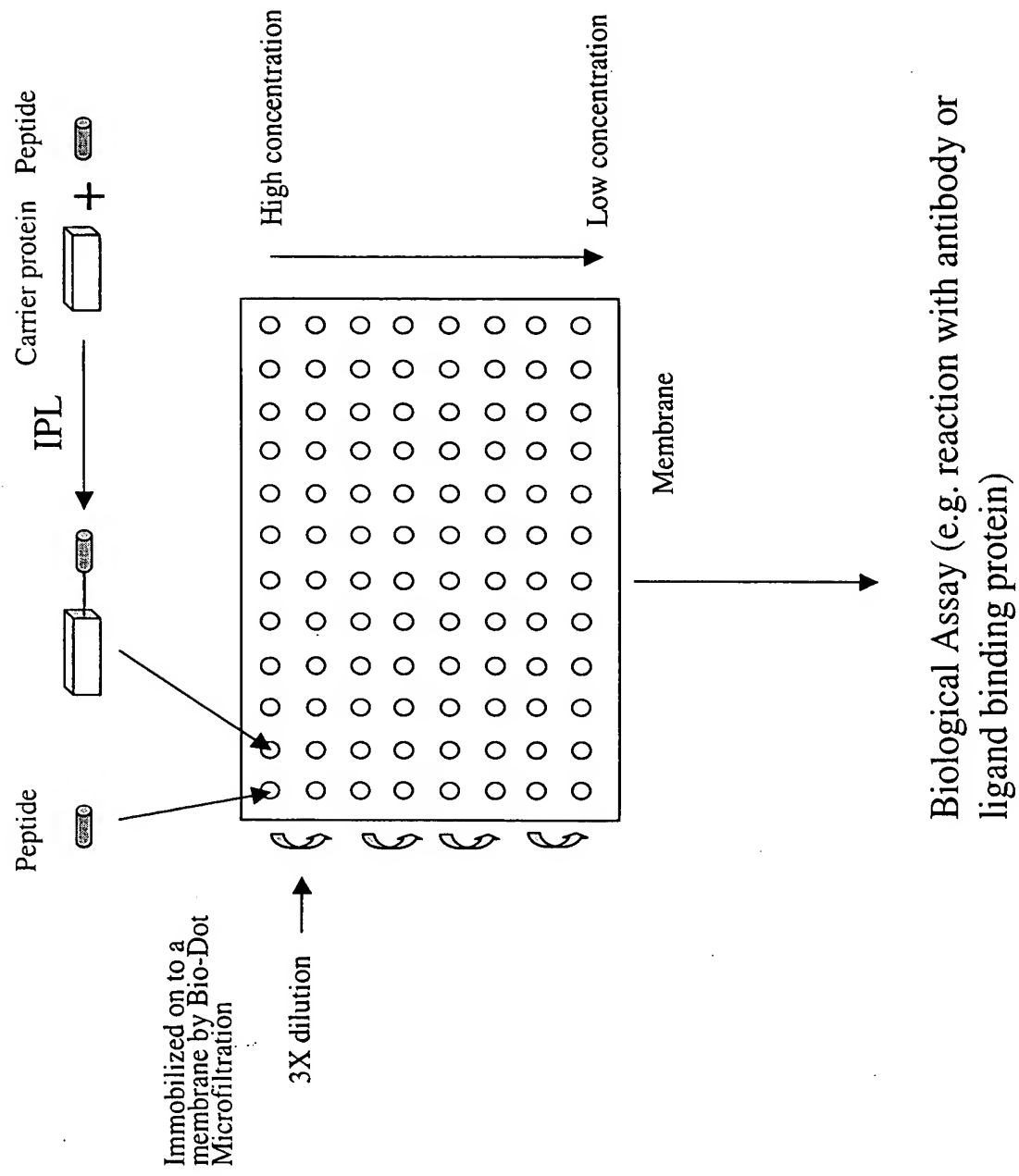


Figure 10. Dot blot analysis of membrane bound peptide substrates by peptide specific antibodies

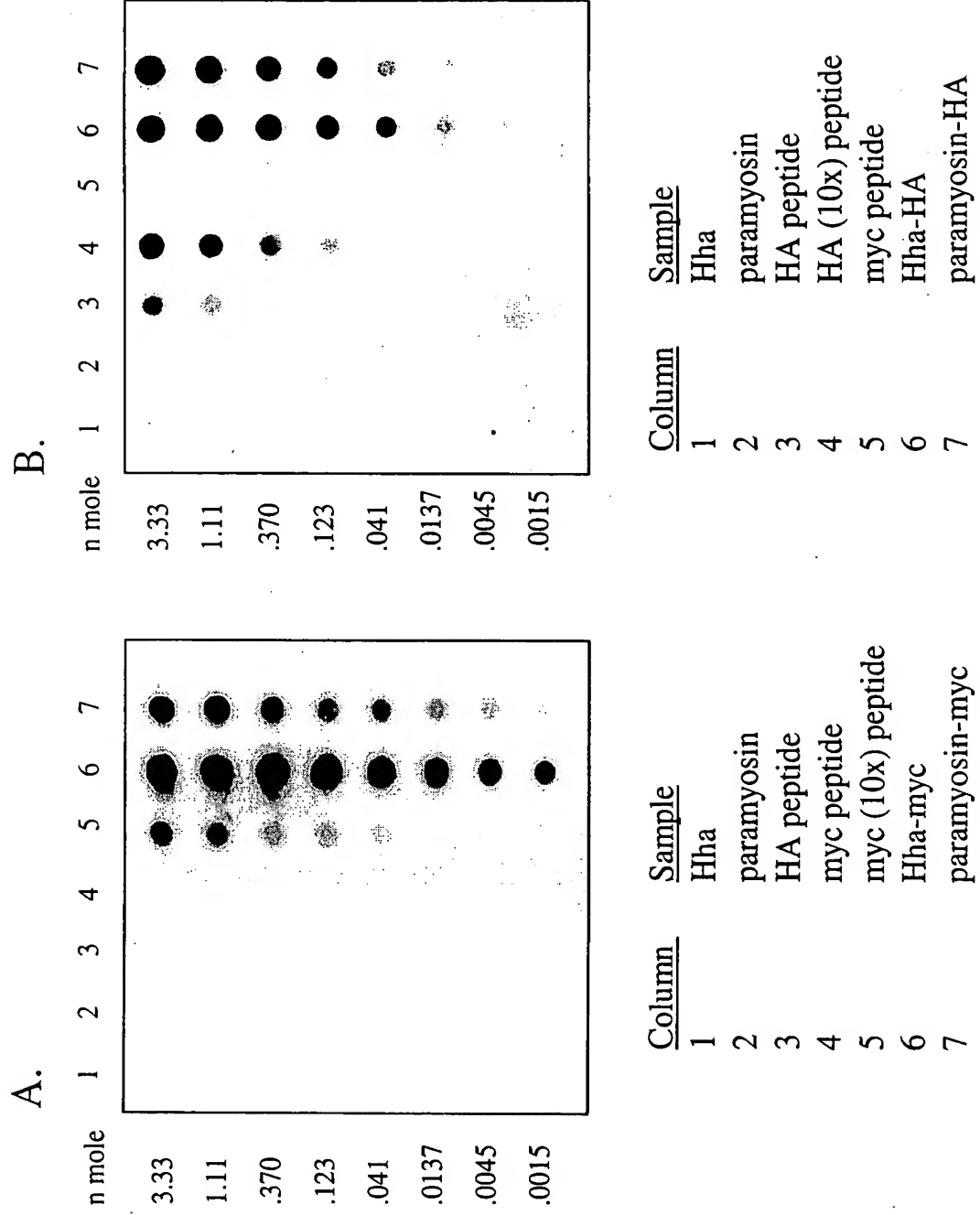
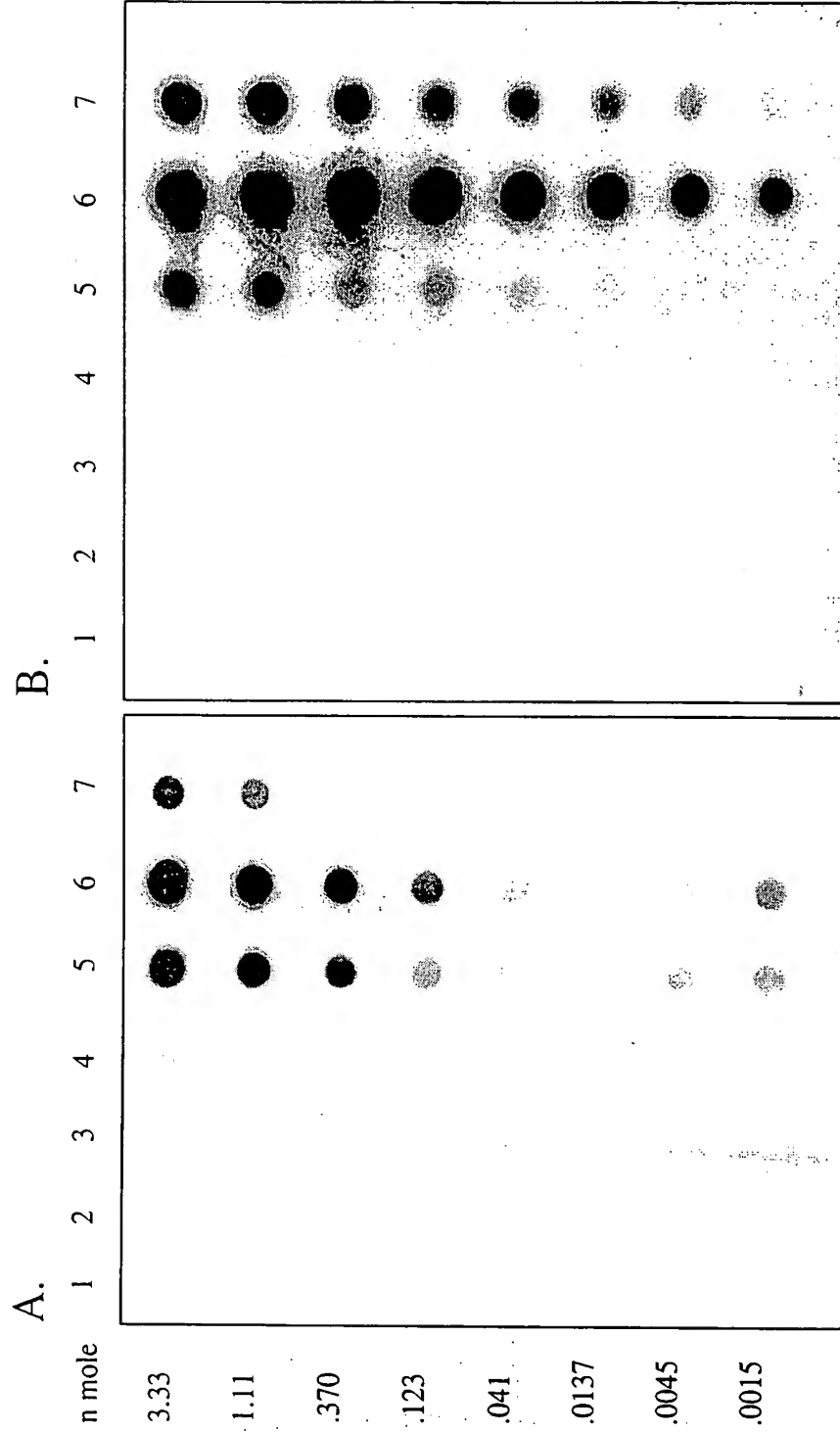


Figure 11. Blotting assays using different membranes



**A. Nitrocellulose 0.2  $\mu$ m**

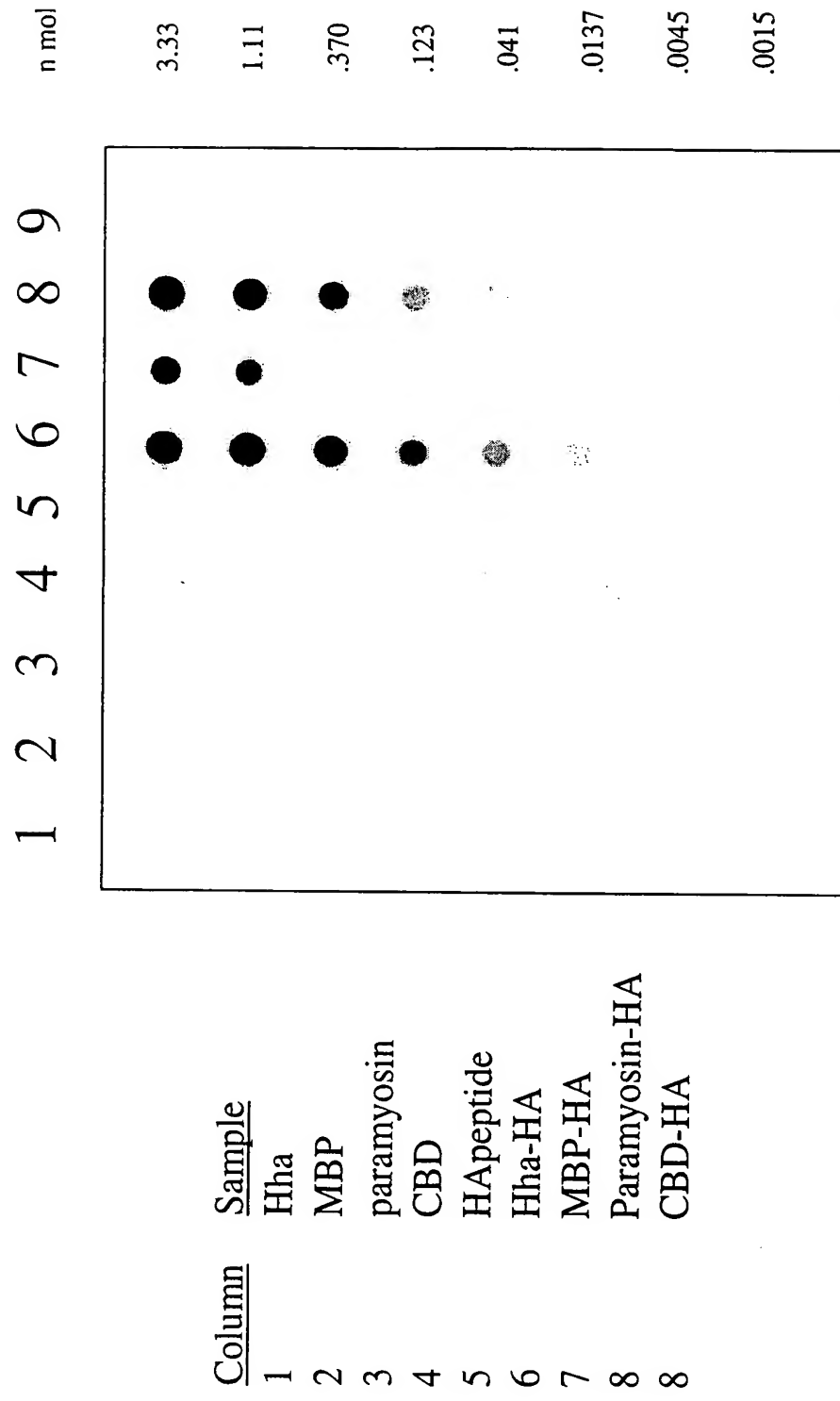
Column	Sample
1	paramyosin
2	HA peptide
3	myc peptide
4	p53 peptide
5	paramyosin-HA
6	paramyosin-myc
7	paramyosin-p53

**B. Nylon 0.2  $\mu$ m**

Column	Sample
1	Hha
2	HA peptide
3	myc peptide
4	p53 peptide
5	Hha-HA
6	Hha-myc
7	Hha-p53

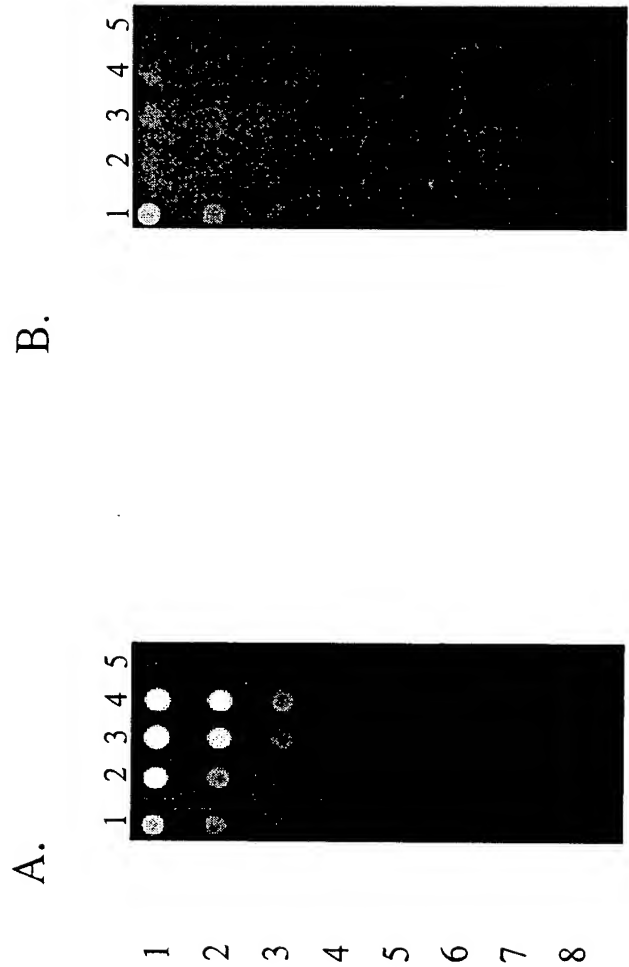
Primary antibodies:  
 anti-HA, 1: 5000  
 anti-myc, 1: 5000  
 anti-p53, 1: 5000

Figure 12. Screening for carrier proteins



Primary antibody: anti-HA, 1:5000.  
0.45  $\mu$ m nitrocellulose membrane

Figure 13. Screening for carrier proteins

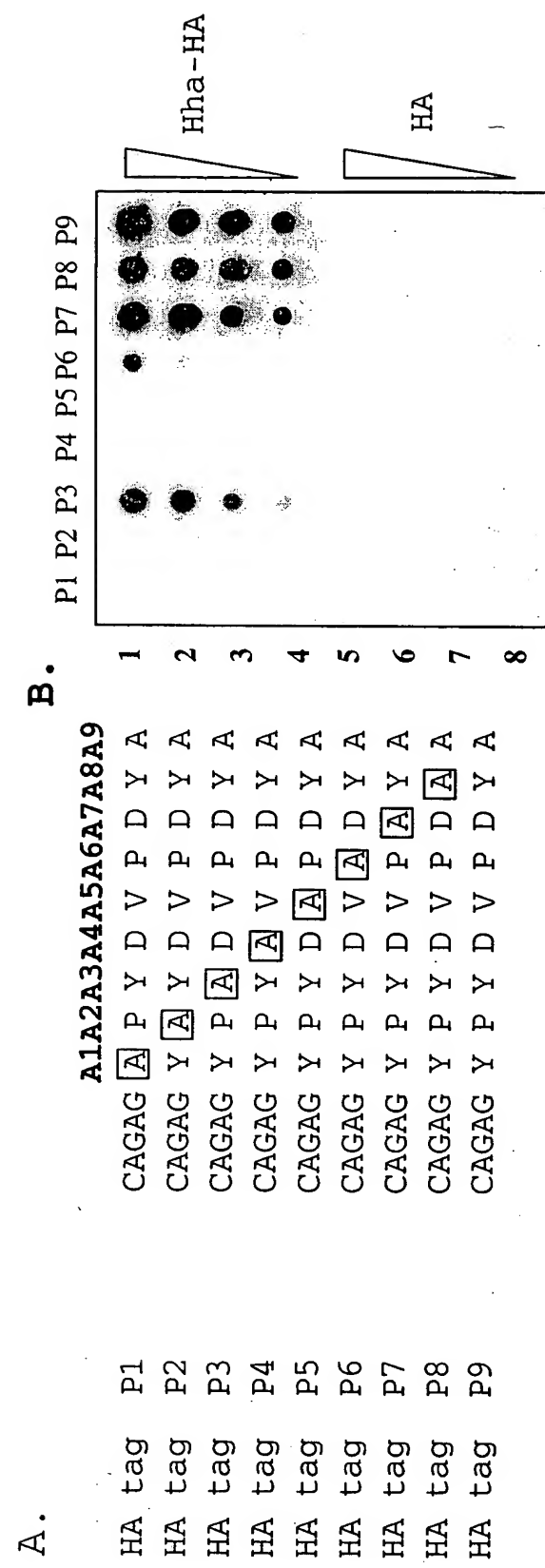


A. before mock western blotting  
B. after mock western blotting  
0.2  $\mu$ m NC membrane

Column	Sample
1	Hha-FluP
2	MBP-FluP
3	paramyosin-FluP
4	CBD-FluP
5	FluP peptide

FluP, CDPEK(Fluorescein)DS

Figure 14. Epitope alanine mapping by  
IPL-enhanced dot blot assay



10  $\mu$ l of 0.5 mM peptide solution was spotted onto 0.45  $\mu$ m nitrocellulose membrane; 3 fold dilution from top to bottom (row 1-4 for HA peptide antigens ligated to carrier protein HhaI or row 5-9 for HA peptides).

Row 1-4: peptide antigens ligated to thioester tagged protein Hha

Row 5-8: peptide antigens

Anti-HA monoclonal antibody (1:5000 dilution, CST)

Figure 15. ELISA assay for ligated HA and myc

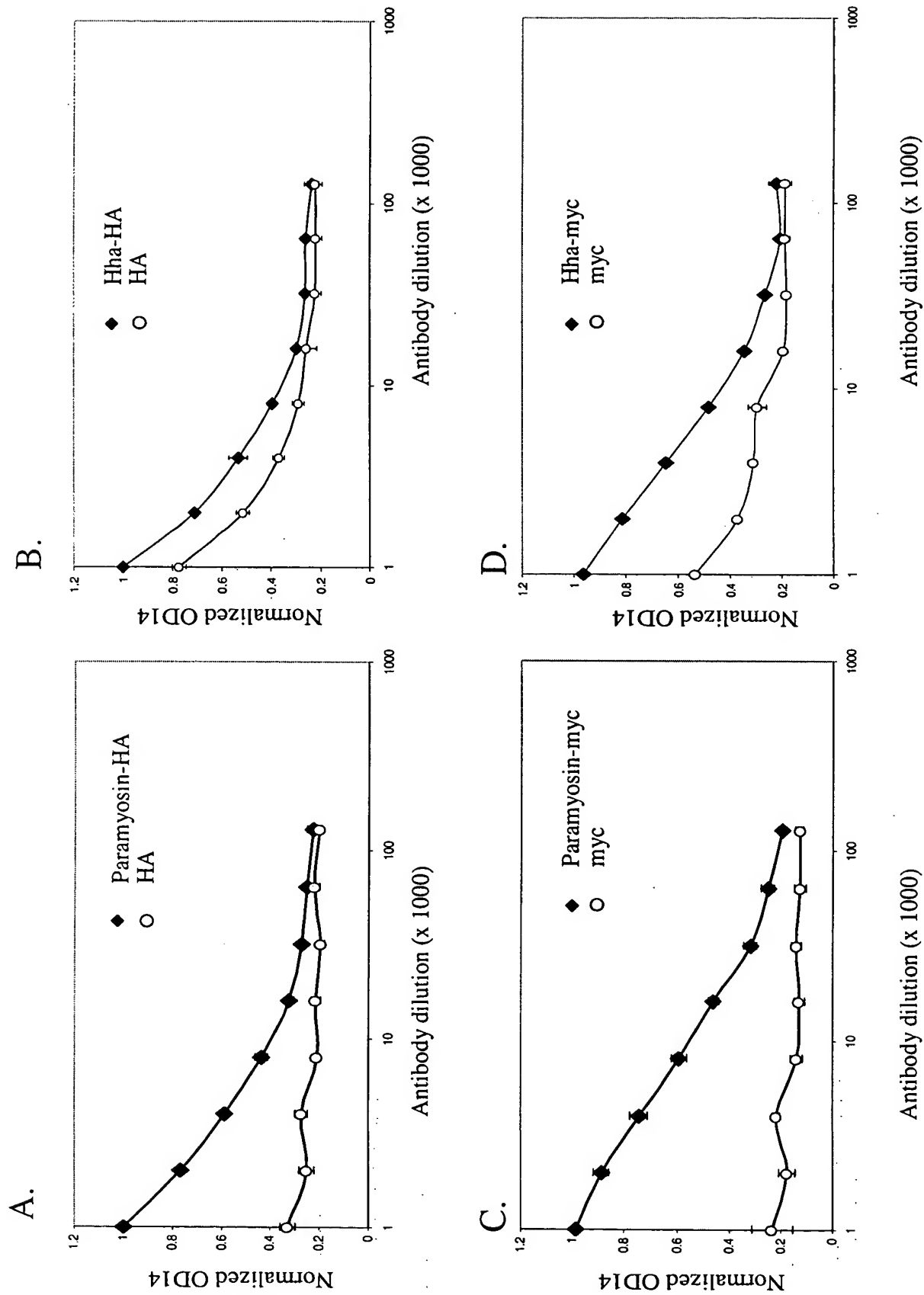


Figure 16. Production of Substrates for Kinase Assay

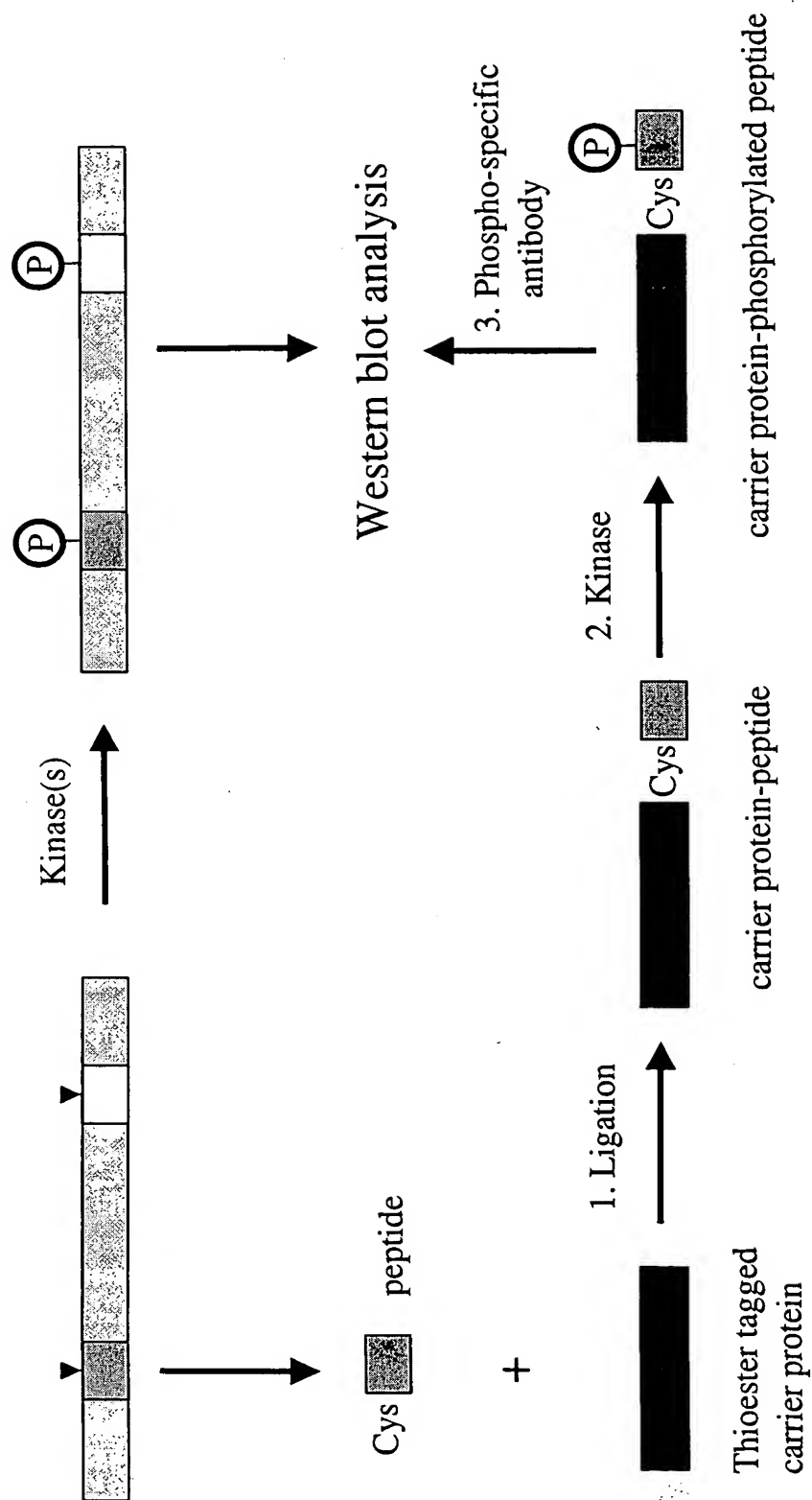




Figure 17. Kinase assay

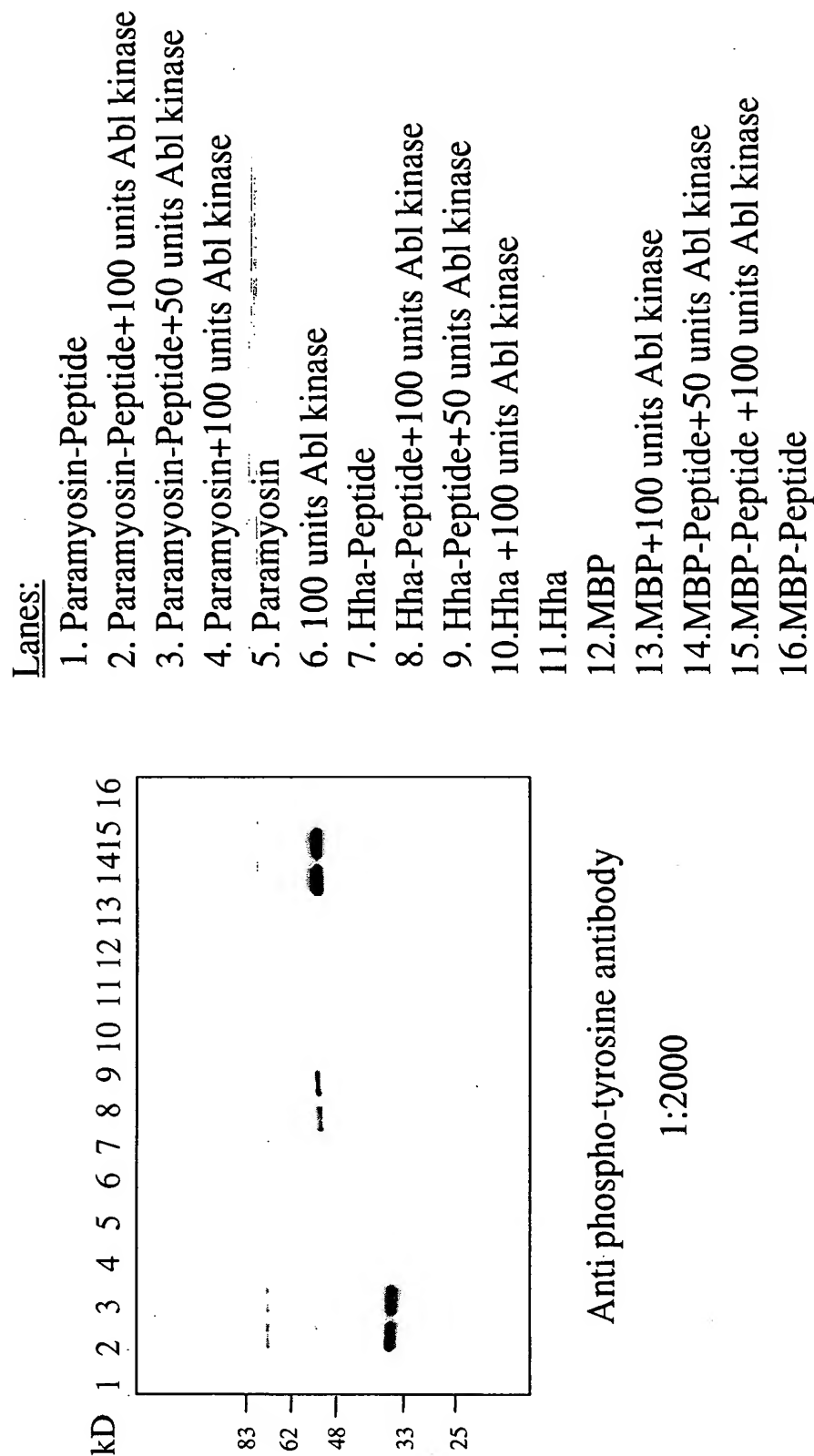
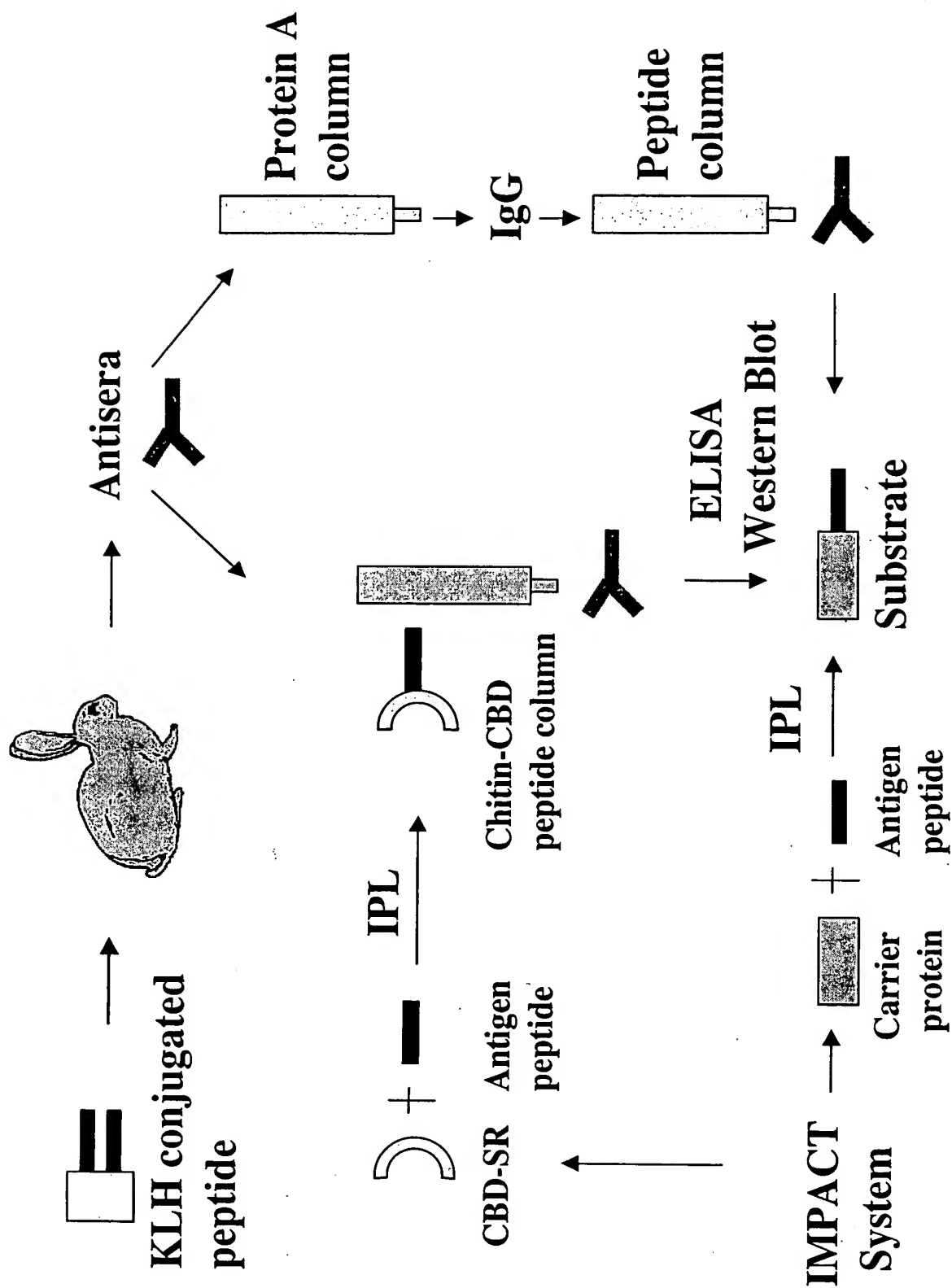


Fig. 18 Use of IPL for Antibody Purification and Characterization



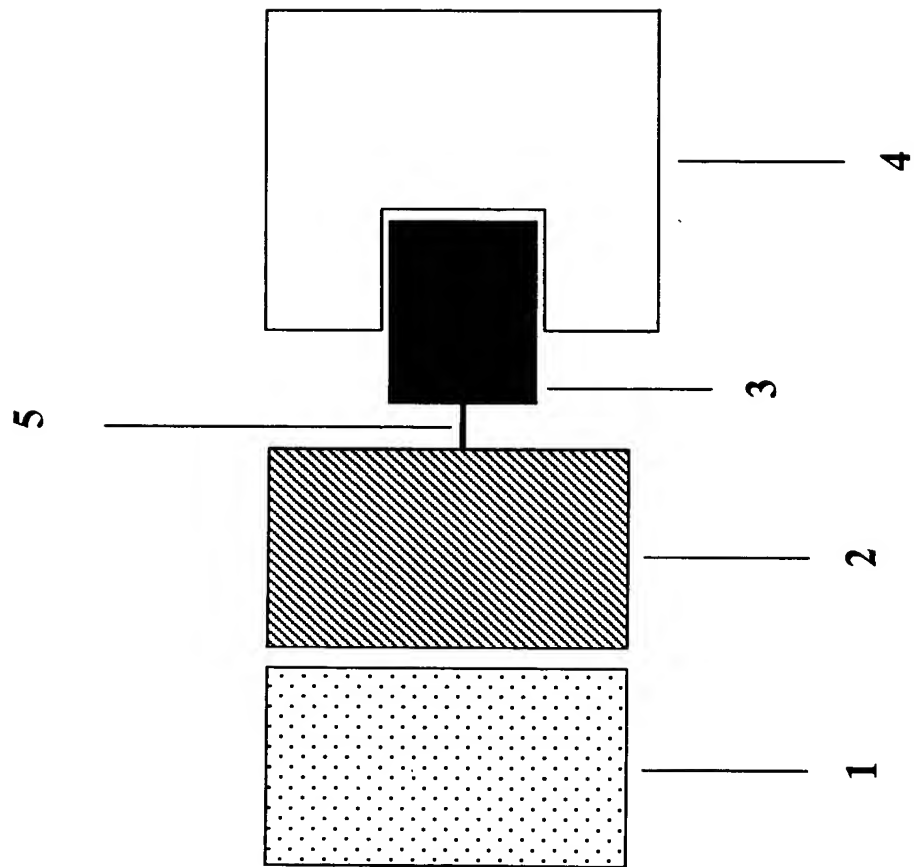


Fig. 19